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The effect of tamoxifen on the electrochemical behavior of Bi^{+3}/Bi on the glassy carbon electrode and its determination via differential pulse anodic stripping voltammetry

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Abstract. The electrochemical behavior of Bi^{+3} ions on the surface of a glassy carbon electrode, in acidic media and in the presence of tamoxifen, was investigated. Cyclic voltammetry, chronoamperometry, differential pulse voltammetry, electrochemical impedance spectroscopy, and scanning electron microscopy with energy-dispersive X-ray spectroscopy were used to find the probable mechanism contributing to the reduction of the peak height of bismuth oxidation with an increase in the concentration of tamoxifen. The obtained results show a slight interaction between the bismuth species and tamoxifen which co-deposit on the surface of glassy carbon electrode. Therefore, the reduction in the peak height of bismuth oxidation as a function of tamoxifen concentration was used to develop a new differential pulse anodic striping voltammetry method for determination of trace amount of tamoxifen. The effects of experimental parameters on the in situ DPASV of Bi^{+3} ions in the presence of tamoxifen shown the optimal conditions as: 2 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH), a deposition potential of -0.5 V, a deposition time of 60 s, and a glassy carbon electrode rotation rate of 300 rpm. The calibration curve was plotted in the range of 0.5 to 6 µg mL^{-1} and the limits of detection and quantitation were calculated to be 3.1×10^{-5} µg mL^{-1} and 1.0×10^{-4} µg mL^{-1}, respectively. The mean, RSD, and relative bias for 0.5 µg mL^{-1} (n=5) were found to be 0.49 µg mL^{-1}, 0.3%, and 2%, respectively. Finally, the proposed method was successfully used for the determination of tamoxifen in serum and pharmaceutical samples.
Keywords: bismuth film electrode; tamoxifen; glassy carbon electrodes; differential pulse anodic stripping voltammetry; serum and pharmaceutical samples.

Resumen. Se investigó el comportamiento electroquímico de los iones Bi^{+3} en la superficie de un electrodo de carbono vítreo, en medios ácidos y en presencia de tamoxifeno. Se utilizaron voltametría cíclica, cronoamperometría, voltametría diferencial de pulso, espectroscopia de impedancia electroquímica y microscopía electrónica de barrido con espectroscopia de rayos X de dispersión de energía con el aumento de la concentración de tamoxifeno. Los resultados obtenidos muestran una ligera interacción entre las especies de bismuto y el tamoxifeno que se depositan conjuntamente en la superficie del electrodo de carbono vítreo. Por lo tanto, la reducción en la altura máxima de la oxidación de bismuto en función de la concentración de tamoxifeno se utilizó para desarrollar un nuevo método de voltametría de banda anódica de pulso diferencial para la determinación de la cantidad de traza de tamoxifeno. Los efectos de los parámetros experimentales en el DPASV *in situ* de iones Bi^{+3} en presencia de tamoxifeno mostraron las condiciones óptimas como: 2 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH), un potencial de deposición de -0.5 V, un tiempo de deposición de 60 s, y una velocidad de rotación del electrodo de carbono vítreo

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de 300 rpm. La curva de calibración se trazó en el rango de 0.5 a 6 μg mL^{-1} y los límites de detección y cuantificación se calcularon en 3.1×10^{-5} μg mL^{-1} y 1.0×10^{-4} μg mL^{-1}, respectivamente. Se encontró que la media, la RSD y el sesgo relativo para 0.5 μg mL^{-1} (n = 5) eran 0.49 μg mL^{-1}, 0.3% y 2%, respectivamente. Finalmente, el método propuesto se utilizó con éxito para la determinación de tamoxifeno en muestras de suero y farmacéuticas.
Palabras clave: electrodo de película de bismuto; tamoxifeno; electrodos de carbono vítreo; Voltametría de extracción anódica de pulso diferencial; Muestras de suero y farmacéuticas.

Introduction

BiFEs have been used in different electroanalytical studies of environmental, clinical, and food samples. The analytical applications of BiFEs generally include the analysis of heavy metal ions [1], inorganic anions [2], and organic compounds [3]. In stripping voltammetry performed with the BiFEs, the analyte species are preconcentrated in an electrolytic or open-circuit manner before anodic, cathodic, and adsorptive stripping measurements. Accordingly, studies refer to different stripping techniques, including linear sweep [4], differential pulse [5] and square wave [6] voltammetry, in the measurement step. However, some studies have reported the stripping analysis of organic compounds, such as acetamiprid [7], imidaclopride [7], sulfadiazine [8], and nitrobenzene [9], using BiFEs. Bismuth film are developed by depositing a thin bismuth film on a suitable substrate material, such as glass carbon [10], carbon paste [11], graphite [12], and screen printed carbon ink [13]. Among these substrates, glass carbon was used electroanalytically more often because of its low background current in comparison to the others [12]. There are three methods for depositing a bismuth film on the surface of a substrate to make a modified electrode. The first method is the *ex situ* electroplating of bismuth ions on the substrate surface [5], the second method is the *in situ* electrodeposition of bismuth ions with an analyte [10], and the third method is to modify the electrode with a bismuth precursor [14].

Tamoxifen is a drug that was discovered in 1967 for treating breast cancer in women and hormone-sensitive cancer in both men and women. The main mechanistic behavior of the drug is that it bonds to the estrogen receptor to prevent the growth of tumors [15]. However, there are many different analytical techniques to measure the content of this drug in blood serum, urine, and pharmaceutical products. Analytical methods, such as spectrophotometry [16], gas chromatography [17], high performance liquid chromatography [18], capillary electrophoresis [19], polarography [20], cyclic voltammetry [21], and differential pulse anodic stripping voltammetry (DPASV) [15], have been used to measure and control the dosage of tamoxifen. Among these techniques, electrochemical methods have been preferred because these methods are simple, rapid, and low cost. As reported in previous studies [15], the tamoxifen tended toward considerable deposition on the surface of glassy carbon in negative potentials less than -0.2 V vs. Ag/AgCl. However, this behavior of tamoxifen has led to the measurement of tamoxifen via stripping techniques with *in situ* bismuth or mercury film formation.

This research studied the electrochemical behavior of bismuth ions in a sulfuric acid medium in both the absence and presence of tamoxifen. Following characterization by cyclic voltammetry (CV), chronoamperometry (CA), and electrochemical impedance spectroscopy (EIS), the behavior of the bismuth ions on the glassy carbon electrode (GCE) surface in the presence of tamoxifen, and following scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) of the tamoxifen-containing bismuth film–modified GCE, a probable mechanism was proposed to understand the effect of tamoxifen on the formation and electrochemical behavior of bismuth film. In the next step, a reduction in the peak height of bismuth oxidation, as a function of the concentration of tamoxifen used in the deposition step, was chosen as the electrochemical signal for developing a new sensitive DPASV method for the determination of tamoxifen. Different parameters, such as the concentration of H_2SO_4, deposition potential, deposition time, and rotation rate, were examined and optimized. Finally, the methodology was calibrated under optimized conditions and subsequently employed to measure the tamoxifen content in biological and pharmaceutical samples.

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Experimental

Materials and Methods

Reagents

The hydrated bi smuth nitrate was purchased from Sigma-Aldrich. The standard solutions of Hg, Cu, Co, Zn, Mn, Fe, Cd, and Cr were prepared from Carl Roth GmbH (Karlsruhe, Germany). The tamoxifen citrate was purchased from Sigma-Aldrich. The other common chemicals, including H_2SO_4 and HCl, were analytical grade and purchased from Merck Co. Aqueous solutions were prepared with ultrapure water (< 0.054 μs cm^{-1}) from the Milli-Q water system.

Apparatus

A potentiostat/galvanostat, model PGSTAT302N (Metrohm-Autolab, Switzerland), equipped with a rotating disk electrode (RDE) and a three-electrode cell was used to record the voltammograms. Ag/AgCl and Pt rods were used as reference and counter electrodes, respectively. A bare GCE or bismuth film–modified GCE served as the working electrode. The CA and EIS experiments were carried out in the same electrochemical cell. SEM-EDS was carried out on a MIRA3 TESCAN machine. A pH meter, model Metrohm 827, and a conductometer, model Metrohm 912 (Switzerland), were used to adjust the pH of the solutions and measure the specific conductivity of the water, respectively.

Preparation of a bismuth film–modified GCE or a tamoxifen-containing bismuth film–modified GCE

A GCE (3 mm diameter) was polished carefully to a mirror-like finish using alumina slurry 0.05 μm and sequentially sonicated for 3 min in ethanol and water. The bismuth film or tamoxifen-containing bismuth film was formed by the *in situ* cathodic deposition of Bi^{3+} ions or by the cathodic co-deposition of Bi^{3+} ions and tamoxifen on the GCE in an H_2SO_4 solution.

Analytical procedure

A 10 mL solution containing 1×10^{-4} mol L^{-1} Bi^{+3} and tamoxifen was transferred to an electrochemical cell. After removing the dissolved oxygen via 30 s N_2 bubbling, the co-deposition of Bi^{+3} and tamoxifen was performed at -0.5 V versus Ag/AgCl in 2 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH). The electrolysis time was 60 s, and after a 10 s rest period, the DPASV was recorded in the range of -0.5 to 0.0 V. The pulse amplitude, interval pulse, and scan rate were 25 mV, 0.5 s, and 10 mV s^{-1}, respectively.

Extraction of tamoxifen from ground tablets

To extract the tamoxifen (20 mg) from tablets, 10 tablets were weighed, and the average mass was calculated. The tablets were ground to a fine homogeneous powder in a mortar. A portion of the finely ground material equivalent to the average mass was dissolved in 6 mL of methanol, stirred in a batch process for 5 min, and filtered using filter paper. Next, 10 μL of the sample was diluted with up to 10 mL of 2 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH) containing 1×10^{-4} mol L^{-1} Bi^{+3}. Subsequently, the DPASV was performed using the proposed method under optimized conductions.

Preparation of spiked serum samples

The serum samples (5 mL) were spiked by tamoxifen solution, and 5 mL of non-spiked serum was selected as the blank. The tamoxifen-spiked serum samples were shaken for 2 min; subsequently, 5 mL of CH_3CN was added to each tamoxifen-spiked serum sample to break the protein-drug binding. This mixture was vortexed for 10 min and then centrifuged at 4000 rpm to separate the precipitated proteins. Subsequently, the supernatant phase was transferred into a 10 mL flask, and the H_2SO_4 concentration was adjusted at 2 mol L^{-1} by adding 2 mL H_2SO_4 (10 mol L^{-1}) containing 5×10^{-4} mol L^{-1} Bi^{+3}. Then the samples were diluted with up to 10 mL Milli-Q water (< 0.05 μs cm^{-1}) and analyzed using the proposed DPASV. The peak height differences of the sample and blank were selected to evaluate the tamoxifen concentration in the spiked-serum samples.

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Results and Discussion

Cyclic voltammetry

First, the electrochemical behavior of the Bi^{+3} ions in a H_2SO_4 solution on the surface of the GCE was studied in the absence and presence of tamoxifen. The acidic medium was preferred because of the low solubility of the bismuth nitrate in aqueous media and the low iR drop of the electrolyte. As seen from Fig. 1, the two peaks in the cathodic and anodic scans appeared at -0.29 V and -0.15 V, respectively. The peak centered at -0.29 V in the cathodic scan is attributed to the reduction of Bi^{+3} ions, while the peak that appeared at -0.15 V is related to the oxidation of the bismuth deposited on the GCE. The presence of tamoxifen in the solution caused a broadening and shifting toward the positive potential of the bismuth's oxidation peak. However, the CV investigations demonstrated the deposition and stripping of the bismuth film in the H_2SO_4 medium in the presence of tamoxifen.

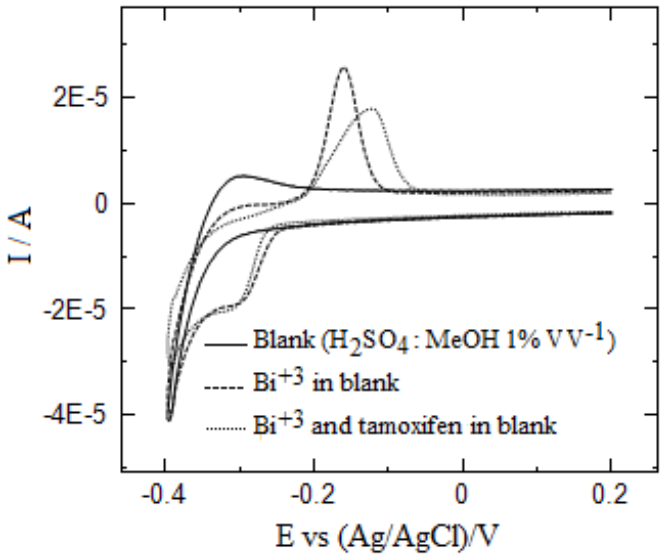

Fig. 1. The CVs of the bare GCE in H_2SO_4 media containing Bi^{+3} ions and Bi^{+3} + tamoxifen. The CV conditions: 0.1 mol L^{-1} H_2SO_4, 1×10^{-4} mol L^{-1} Bi^{+3}, and a scan rate of 100 mV s^{-1}.

Mechanistic studies

Chronoamperometry, cyclic voltammetry at different scan rates and anodic/cathodic differential pulse voltammetry were investigated in details in supplementary information.

EIS behavior of bare and modified GCEs

To better understand the reduction mechanism of Bi^{+3} ions in the presence of tamoxifen, EIS studies were conducted. In the first experiment, the EIS behavior of a bare GCE in a Bi^{+3} and tamoxifen-containing Bi^{+3} solution at -0.5 V was determined. In the second experiment, the EIS behavior of the bismuth film–modified GCE and the tamoxifen-containing bismuth film–modified GCE, which were prepared by the deposition of Bi^{+3} ions and Bi^{+3} ions and tamoxifen respectively at -0.5 V (60 s), were recorded at the oxidation potential of the bismuth at -0.15 V.

As seen from Fig. 2a, for both tamoxifen-free and tamoxifen-containing Bi^{+3} solutions, the Nyquist spectrum consisted of a semicircle, which presents a charge-transfer step at higher frequencies, and a linear part, which reflects a diffusion step at lower frequencies. However, comparing the Nyquist spectra of the Bi^{+3} solutions at -0.5 V demonstrates that the presence of tamoxifen affects the charge-transfer step at high frequencies and the diffusion of Bi^{+3} ions at low frequencies, as the amperometery results show. The Bode modulus impedance and Bode phase spectra of both Bi^{+3} solutions (Fig. 2b) represent different behaviors in high, middle and low frequencies. As seen, in the high frequencies region, the phase angle tends to zero, and the impedance value reaches a horizontal amplitude, with increasing frequency. These two EIS responses indicate the resistance behavior of the electrochemical system at high frequencies, as uncompensated resistance that a large part of it comes from the electrolyte resistance. The middle segment represents a linear relation between the impedance values and frequencies with a slope close to -1, and a phase angle that tends to -90. The EIS study in the middle frequencies can

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present a combined equivalent circuit of capacitances and resistances, corresponding to the electrochemical and physical phenomena. In low frequencies, the phase angle reaches to zero, and the impedance values are approximately independent of the frequency. A horizontal amplitude, and the phase angle zero, can be attributed to the overall resistance of the system, which include the charge-transfer resistance [15]. As shown in Fig. 2b, the charge-transfer resistance of the GCE in tamoxifen-containing solution is greater than the tamoxifen-free solution. Thus, the obtained EIS results may indicate the resistance behavior of the co-deposited tamoxifen against the reduction and deposition of the Bi^{+3} ions at -0.5 V.

The Nyquist spectra of the bismuth film–modified and tamoxifen-containing bismuth film–modified GCEs, in a fresh H_2SO_4 solution, and under static conditions at -0.15 V, shown in Fig. 2c. The Nyquist spectrum of the bismuth film–modified GCE, which shows almost a linear behavior, may indicate the limitation of the bismuth film oxidation by diffusion of the Bi^{+3} ions from electrode surface to the H_2SO_4 electrolyte. The Nyquist plot, related to the tamoxifen-containing bismuth film–modified GCE, consisted of a semicircle and a straight line. Here, the semicircle, which illustrates the charge-transfer resistance, is affected by the straight line. This observation, can be an evidence that the oxidation of bismuth contained in the tamoxifen-containing bismuth film, is controlled by both the charge-transfer process and the diffusion of Bi^{+3} ions at high frequencies region. This result, means that the tamoxifen molecules can co-deposit with Bi^{+3} ions, on the surface of GCE in an H_2SO_4 solution, and subsequently affect the charge transfer step of the bismuth film oxidation at high frequencies. The Bode modulus impedance and Bode phase spectra of both film-modified GCEs were also shown in Fig. 2d. The comparison of these EIS responses, presents that the impedance value and phase angle for tamoxifen-containing bismuth film-modified GCE, at all frequencies, are higher and lower than the bismuth film-modified GCE values, respectively. It means that the ingress of tamoxifen molecules in the bismuth film provide additional restrictions for bismuth oxidation.

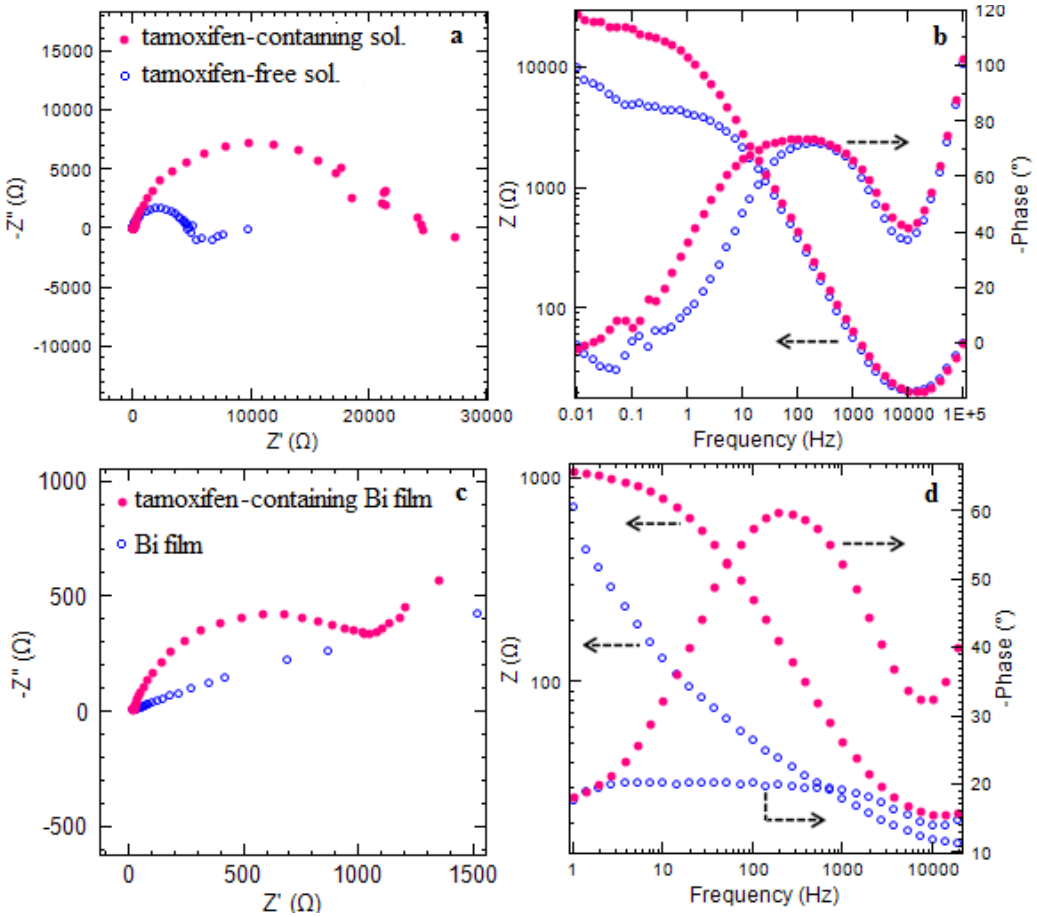

Fig. 2. (a) Nyquist spectra and (b) Bode modulus/Bode phase plots of bare GCE, in 0.1 mol L^{-1} H_2SO_4 containing 1 × 10^{-4} mol L^{-1} Bi^{+3} (hollow circle), and in 0.1 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH) containing 1 × 10^{-4} mol L^{-1} Bi^{+3} + 5 µg mL^{-1} tamoxifen (solid circle). (c) Nyquist spectra and (d) Bode

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modulus/Bode phase plots of bismuth film–modified GCE (hollow circle) and tamoxifen-containing bismuth film–modified GCE (solid circle) in 0.1 mol L^{-1} H_2SO_4. Conditions: AC amplitude 0.01 V, applied frequencies 0.01 Hz to 100 KHz, and potential -0.5 V.

SEM images

In order to investigate the differences in the bismuth film deposited on the GCE in the absence and in the presence of tamoxifen, SEM and EDS analyses were also performed. The obtained results are shown in Fig. 3. The image of the film deposited in the presence of tamoxifen (Fig. 3c), in comparison with the film deposited in the absence of tamoxifen (Fig. 3b), shows large bismuth particles on the surface of the GCE. This is due to the occupation of some of the electrochemical sites of the GCE by the tamoxifen, which limits the available surface for the deposition of bismuth ions. Therefore, the deposition of Bi^{+3} ions in the presence of tamoxifen at a constant step potential results in the formation of larger particles on the GCE surface. This image of the bismuth film, formed in an H_2SO_4 solution free from tamoxifen (Fig. 3b), shows small particles that are very close to each other, so that some areas create complex aggregations. The EDS results of both films are presented in Fig. 3d and e. The results (Fig. 3e) show an increase in the carbon peak height and a decrease in the bismuth peak height with the ingress of tamoxifen molecules into the bismuth film deposited on the GCE. These peak-height changes provide a quantitative proof of the formation of a tamoxifen-containing bismuth film on the surface of the GCE in the presence of tamoxifen.

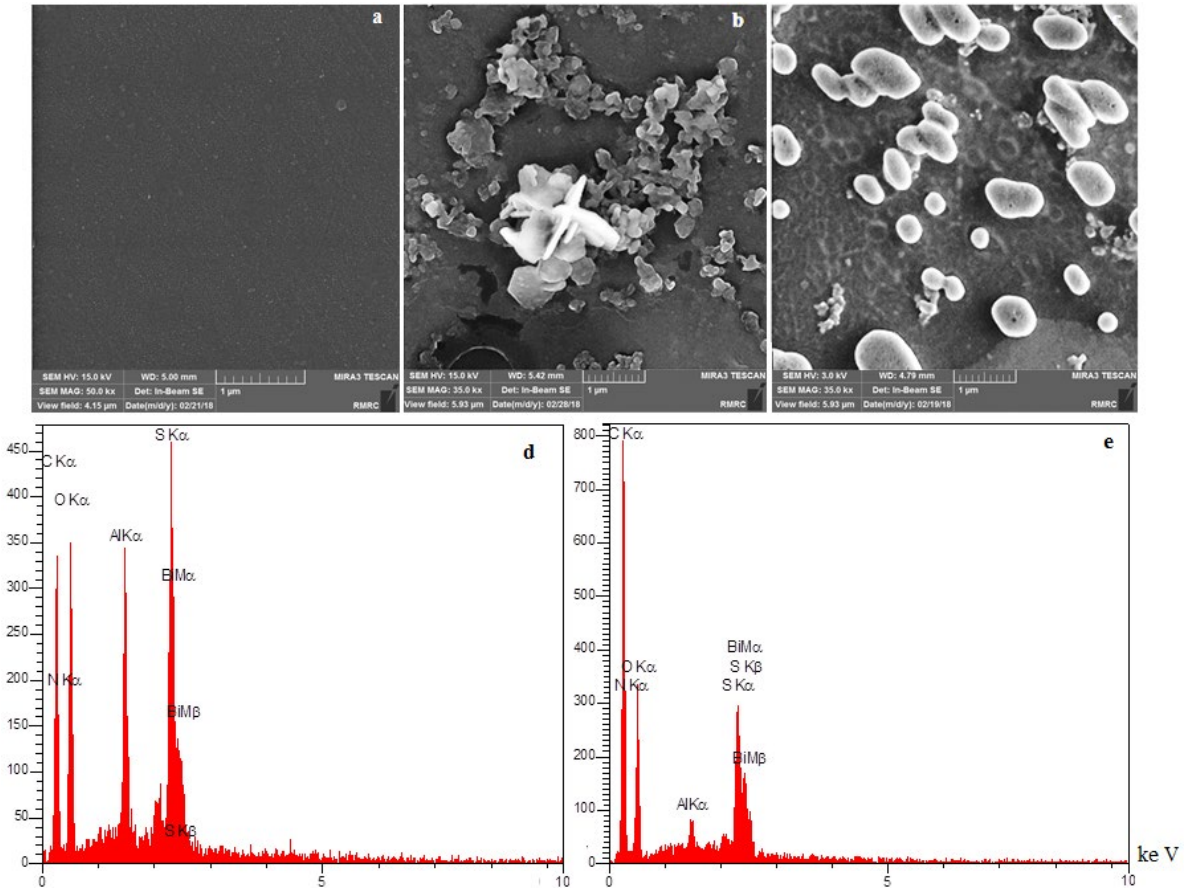

Fig. 3. The SEM images of the (a) bare GCE, (b) bismuth film modified-GCE, and (c) tamoxifen-containing bismuth film–modified GCE, and the EDS results of the (d) bismuth film–modified GCE and (e) tamoxifen-containing bismuth film–modified GCE. The conditions of film formation: 0.1 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH), 1 × 10^{-4} mol L^{-1} Bi^{+3}, 5 µg mL^{-1} tamoxifen, a deposition potential of -0.5 V, a deposition time of 60 s, and a GCE rotation rate of 300 rpm.

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The effect of different parameters on the reduction of the peak height of bismuth oxidation

All experiments on the optimization of the different parameters for the reduction of the peak height of bismuth oxidation using the *ex situ* stripping method were carried out under conditions of 1×10^{-4} mol L^{-1} Bi^{+3}, 5 µg mL^{-1} tamoxifen, electrolyte 0.1 mol L^{-1} H_2SO_4 (1% v v^{-1} MeOH), a deposition potential of -0.5 V, a deposition time of 60 s, a stripping rate of 300 rpm, and a sample volume of 10 mL, except when each factor was under investigation.

The effect of acid and its concentration

The effect of HCl and H_2SO_4, a common inorganic acid, on the bismuth peak-height reduction in the presence of tamoxifen was evaluated. A range of 0.01 mol L^{-1} to 4 mol L^{-1} was chosen for the range of acid concentration. To perform this investigation, the *ex situ* DPASV of the bismuth films prepared in the absence and presence of tamoxifen was performed and the results recorded. Subsequently, the peak-height difference was plotted against the acid concentration. It was observed that the peak potential of bismuth oxidation shifts to a more negative potential with an increase in the acid concentration. The major reasons for this may be the low iR drop of the electrolyte and the fast kinetics of the hydrogen evolution at the surface of the counter electrode. The peak height differences against the acid concentration, as plotted in Fig. S5a, show that the maximum decreases are achieved by 2 mol L^{-1} H_2SO_4. Therefore, 2 mol L^{-1} H_2SO_4 was chosen as the best electrolyte for developing a sensitive DPASV method for the determination of tamoxifen.

Effect of the deposition potential

As observed in the CV results, the Bi^{+3} ions can be deposited at a potential of less than -0.4 V. Hence, a set of experiments were carried out in the deposition potential range of -0.2 to -0.8 V. The decrease of peak height, as a function of deposition potential, was plotted in Fig. S5b. As demonstrated, the highest peak-height difference was obtained for the deposition potential -0.5 V. This is because of the significant contribution of the hydrogen evolution reaction at a more negative potential, which prevents the co-deposition of Bi^{+3} ions and tamoxifen. It is well-known that the potentials greater than the reduction potential of the Bi^{+3} ions is not sufficient to deposit both the Bi^{+3} ions and for the cathodic adsorption of tamoxifen [15]. The deposition potential -0.5 V, which was applied in the previous experiments, was selected as the optimal value in the next experiments.

Effect of the deposition time

One of the most important parameters in the stripping experiments is deposition time. In this step of the optimization, the deposition time was varied in the range of 30–240 s. The peak-height differences as a function of deposition time are shown in Fig. S5c. It is obvious that the function reaches the maximum value at deposition time 60 s. This may be due to decreased deposition of Bi^{+3} and tamoxifen at times less than 60 s and the removal of some co-deposited tamoxifen molecules by hydrogen attack at times greater than 60 s. Based on this, 60 s was selected as the optimal value for the deposition time in the next experiments.

Effect of the stirring rate

To evaluate the effect of the convection-enhanced mass transfer of the Bi^{+3} ions and tamoxifen on the peak-height difference, the rotation rate of the working electrode, as a driving force of convection, was varied in the range of 100–500 rpm. The ΔI_{peak} versus the rotation rate was plotted and is shown in Fig. S5d. As can be seen, the maximum ΔI_{peak} is achieved at 300 rpm, which was also selected in the previous experiments. Based on this, a rotation rate of 300 rpm was chosen as the best value for performing the next experiments.

Comparing in situ and ex situ stripping of tamoxifen-containing bismuth film

To enhance the sensitivity of the bismuth oxidation peak with the presence of tamoxifen, *in situ* stripping was also carried out. The obtained results for *ex situ* and *in situ* are summarized in Fig. S6. In contrast to the *ex situ* stripping, the *in situ* experiment showed a greater decrease in the peak height of the bismuth oxidation (Fig. S6b and b*). Therefore, the next experiments were performed using the *in situ* mode.

Effect of interferences on the bismuth peak-height difference (ΔI)

To evaluate the selectivity of the bismuth peak height's reduction in the presence of the tamoxifen analyte, the *in situ* anodic stripping voltammetry was also carried out in the presence of a multi-functional polymeric ligand, poly para-aminophenol [22]. This multi-functional ligand was employed to determine whether other organics can

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interfere with the reduction of the bismuth oxidation peak by tamoxifen. As seen from Fig. S7a, the dotted line shows that no significant change was observed in the peak-height reduction of the bismuth film due to poly para-aminophenol. The effect of cationic species such as Cu^{+2}, Zn^{+2}, Co^{+2}, Mn^{+2}, Fe^{+2}, Cd^{+2}, Cr^{+2}, and Ni^{+2} were also evaluated at the level of 100 μg L^{-1}. The obtained results (Fig. S7b) demonstrate that there was not a significant difference between the presence of the metal cations and the absence of the metal cations. It is valuable to state that the presence of different drugs such as; ascorbic acid, lactic acid, acetaminophen, albumin, uric acid and keratin were examined at the level of 50 μg mL^{-1}, and shown no significant interferences in measurement of 5 μg mL^{-1} tamoxifen. The proposed method can thus successfully be employ for the determination of tamoxifen using the *in situ* stripping voltammetry for Bi^{+3} ions in the presence of tamoxifen.

Calibration of the proposed method and merit figures

To perform the calibration of the *in situ* DPASV of bismuth, the tamoxifen concentration range was chosen as 0.5–6 μg mL^{-1}. The obtained voltammograms for different concentrations of tamoxifen are presented in Fig. 4. As demonstrated in Fig. 4, inset, a linear relation was observed between ΔI_P and tamoxifen concentration with a significant correlation. The analytical sensitivity is sufficient to measure trace tamoxifen in biological and pharmaceutical samples. The LOD and LOQ were found to be 3.1 × 10^{-5} μg mL^{-1} and 1.0 × 10^{-4} μg mL^{-1}, respectively. To estimate the validity of the proposed method, five standard samples with a concentration of 0.5 μg mL^{-1} were analyzed. The mean, relative standard deviation (RSD), and trueness (as relative bias) were calculated to be 0.49 μg mL^{-1}, 0.3%, and 2%, respectively. These merit figures are sufficiently reliable to employ this methodology as a valid analytical method for the determination of tamoxifen medication.

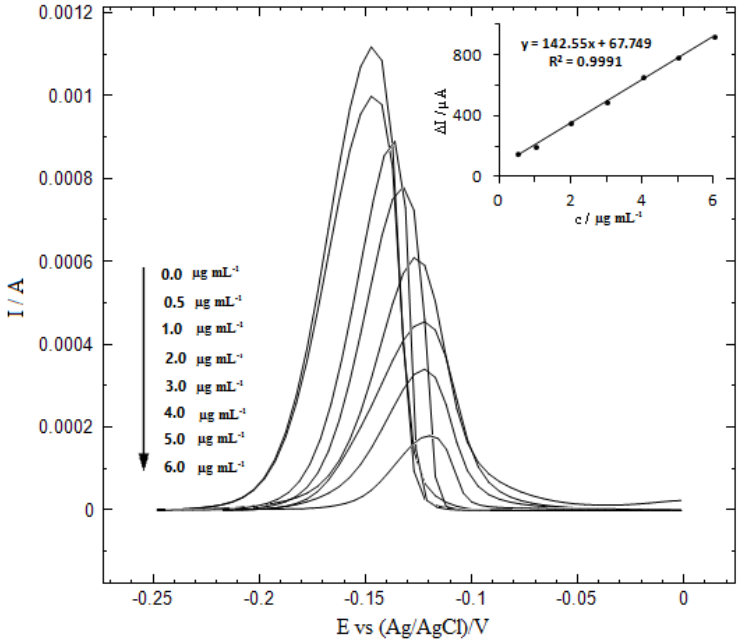

Fig. 4. The obtained voltamograms for the *in situ* stripping voltammetry of Bi^{+3} ions in the presence of different concentrations of tamoxifen as well as the calibration curve (inset).

Application of the methodology in pharmaceutical and biological analysis

To employ the methodology in pharmaceutical analysis, tablet samples from Iran Hormone Co. and Astra Zeneca GmbH Co. were prepared for analysis according to the instructions described in Sec. 2.5. The obtained results are shown in Table 1, and the obtained recoveries present sufficient quality for the application of the proposed method in pharmaceutical analysis. In order to measure the tamoxifen content in two tamoxifen spiked-serum samples (2 μg mL^{-1} and 5 μg mL^{-1}), the samples were prepared and subsequently treated to break the bond between the tamoxifen and the serum's proteins (Sec. 2.6). The calculated concentrations and recoveries, according to the calibration and dilution factors, are presented in Table 1. As seen from Table 1, this methodology has

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sufficient merit to replace high performance liquid chromatography (HPLC) and other analytical techniques for the determination of tamoxifen medication in pharmaceutical and biological samples.

Table 1. Analysis of the real samples by the proposed method.

NO.	sample	source	tamoxifen		Recovery (%)
			Content (mg)	Spiked (µg mL-1)	
1	tablet	Iran Hormone Co.	20	-	97.3
2	tablet	Astra Zeneca GmbH Co.	20	-	97.7
3	serum	Young woman [a]	-	2	103.1
4	serum	Old woman [b]	-	5	102.8

a: 20 years' old
b: 55 years' old

Comparison with other reported methods

Table 2 compares the analytical features of the proposed methodology with those reported for other analytical techniques for the determination of tamoxifen. It is obvious from Table 2 that the proposed DPASV, based on the *in situ* bismuth film formation in an H_2SO_4 solution containing tamoxifen analyte, has lowe detection and quantitation limits, and RSD, compared to the other techniques.

Table 2. Comparison the proposed method with other techniques.

Method	LOD /µg mL^{-1}	LOQ /µg mL^{-1}	RSD (%)	Ref.
Nonaqueous capillary zone electrophoresis	3×10^{-3}	1×10^{-2}	2.8	[23]
Spectrophotometric	8.8×10^{-2}	0.26	< 2%	[24]
Micellar Liquid chromatography	7×10^{-2}	0.2	< 1.5%	[25]
HPLC	9×10^{-4}	3×10^{-3}	8.78	[26]
Flow-injection potentiometric detector	15.6	-	1.81	[27]
Adsorptive potentiometric striping	148.6	-	5.3	[28]
Cyclic voltammetry (CV)	9×10^{-5}	2.9×10^{-4}	0.45	[21]
Square wave voltammetry (SWV)	3×10^{-3}	9×10^{-3}	0.72	[29]
Differential pulse anodic voltammetry (DPAV)	8×10^{-3}	2.5×10^{-2}	2.3	[15]
Proposed method	3.1×10^{-5}	1.0×10^{-4}	0.3[a]	

a: n=5 and tamoxifen concentration; 0.5 µg mL^{-1}

Conclusions

Modification of the GCE using *in situ* and *ex situ* film formation in an H_2SO_4 solution containing tamoxifen was performed. Electrochemical investigations, such as CV, CA, EIS, and DPV, and SEM confirmed the surface-controlled co-deposition of tamoxifen and bismuth ions. It was also observed that this co-deposition can selectively reduce the peak height of the oxidation of the bismuth film in the stripping stage. Additional electrochemical studies qualitatively proved the interaction between Bi^{+3} ions and tamoxifen in an H_2SO_4 solution, as well as between bismuth atoms and tamoxifen in the film deposited on the surface of the GCE. Therefore, this behavior led us to develop a new electrochemical method using *in situ* bismuth film formation in a tamoxifen-containing H_2SO_4 solution for measuring trace amounts of the tamoxifen drug. The different parameters for the DPASV of in situ bismuth film formation in the presence of tamoxifen were optimized, and the influence of probable interferences was examined. However, the proposed DPASV was calibrated and successfully employed for the determination of tamoxifen in different samples.

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